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The NCI In Vitro Anticancer Drug Discovery Screen

Concept, Implementation, and Operation, 1985-1995

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CONTENTS

1. INTRODUCTION

1.1. The Background

Since 1955, the US National Cancer Institute (NCI) has provided screening support to cancer researchers worldwide. Until 1985, the NCI screening program and the selection of compounds for further preclinical and clinical development under NCI auspices had relied predominantly on the in vivo L1210 and P388 murine leukemias and certain other transplantable tumor models (*1*). From 1975-1985, the in vivo P388 mouse leukemia model was used almost exclusively as the initial or primary screen. With few exceptions, agents that showed minimal or no activity in the P388 system were not selected by the NCI for further evaluation in other tumor models or alternative screens. Most of the available clinical anticancer agents are active in the P388 system; however, most were discovered prior to 1975 or by observations initially in test systems other than the NCI-operated P388 primary screen.

1.2. The Concept

In June of 1984, the author presented to the NCI Division of Cancer Treatment's Board of Scientific Counselors (BSC) a preliminary concept of a so-called disease oriented in vitro primary anticancer drug screen as a potential replacement to the P388 in vivo primary screen. Although the new concept was greeted initially with limited enthusiasm, the presenter nonetheless was encouraged to return to the subsequent fall meeting of the BSC with a more fully developed concept for further review and discussion. The new screening model that was proposed to the Board at the October 1984 meeting (*2,3*) comprised the essence of the

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growth-inhibitory effects against each cell line comprising the panel. A secondary stage of screening on selected compounds would be performed in vivo in xenograft models using a subset of the cell lines found to be sensitive in the in vitro screen.

Although simple in concept, the development and implementation of the new in vitro NCI primary screen presented unprecedented challenges. Nevertheless, the feasibility of the pilot screen was firmly established by mid-1989, and in April 1990 the screen was established in fully operational status. Beginning in 1990, samples were screened at a rate of approx 20,000/yr, with the input divided about equally between pure compounds submitted to the NCI, and extracts or fractions thereof originating primarily from the NCI natural products repository. In its first operational configuration, the cell line panel consisted of a total of 60 human tumor cell lines arranged in subpanels representing diverse histologies.

2. IMPLEMENTATION 1985-1990

2.1. Overview and Oversight

Efforts to establish the feasibility of the proposed new in vitro primary screen were focused initially on three main fronts: investigation of various alternative assays of in vitro drug sensitivity (*16-23*); development of the cell line panel (*23-25*); and information technology (*6,26,27*). The technical challenges to implementation of such a screen, requiring on the order of 10-20 million individual cell culture assays/yr to achieve the scope of operations envisaged, were daunting. Also, many critical choices had to be made with respect to the design principles of the screening model, which would, in turn, have profound impact on screening operational logistics as well as the nature of the data output and potential utility thereof. To assist us in making such critical decisions, as well as to provide in-depth, regular oversight of implementation of the new program, we organized an internationally comprised, external "Ad Hoc Review Committee for the NCI In Vitro/In Vivo Disease-Oriented Screening Project" under the chairmanship of Kenneth R. Harrap. Participants (non-NCI) in one or more meetings of that key committee (the "Harrap committee") during its 1985-1990 existence are named in Appendix B. The Harrap committee, or a subcommittee thereof, met at least once annually with NCI staff for detailed discussions, debate, and critique of the new program. The 1987 meeting of the Harrap committee was combined with a second workshop entitled "Selection, Characterization, and Quality Control of Human Tumor Cell-Lines for the NCI's New Drug Screening Program," jointly organized and held in Bethesda, MD on May 27-28, 1987. Participants in that meeting are named in Appendix C. Verbatim transcripts of all these meetings (*14,28-32*) provide interesting documentation of the progress, as well as the challenging technical problems encountered, during the 1985-1990 period. In addition to the Harrap committee reviews, the development of the new screen was also reviewed periodically during regular meetings of the full membership of the BSC and likewise by the National Cancer Advisory Board (NCAB).

2.2. In Vitro Microculture Assays of Cell Growth and Viability

Three alternative assays for cellular growth and viability for possible use in the new primary screen were extensively investigated (*16-23*). Two were metabolic assays (*16,18*); in both, the cellular reduction of a colorless tetrazolium salt (MTT or XTT) yielded a colored

formazan in proportion to viable cell number. The formazans could be measured conveniently in an automated colorimeter.

The development of the XTT tetrazolium assay (*18*) was stimulated by the desire to simplify further the MTT procedure by eliminating an aspiration/solubilization step; the reduction of MTT yielded an insoluble formazan, which had to be dissolved in dimethylsulfoxide prior to colorimetry. In contrast, the XTT reagent (*17*) was metabolized by viable cells directly to a water-soluble formazan, allowing the immediate reading of optical density in the culture wells without further processing. Although simple and convenient, the XTT procedure gave relatively high background readings low "signal-to-noise" ratio). XTT also shared with MTT the feature of an unstable (i.e., time critical) end point, compromising the potential use of either of the tetrazolium assays in a high-flux antitumor screen employing a large panel of cell lines.

Although the XTT tetrazolium assay ultimately was not adopted for the anticancer screen, it did prove to have an immensely valuable application in the DTP-NCI high flux anti-human immunodeficiency virus (HIV) drug discovery screen. The concept and development of the anti-HIV screen was first proposed by the author to NCI management in November 1986, was subsequently presented to and formally approved by the BSC in February 1987, and was pursued thereafter by DTP in parallel to the anticancer screen (*33,34*). We also organized a separate external Ad Hoc Advisory Committee, initially under the chairmanship of Dani P. Bolognasi and later under William M. Mitchell, to provide critique and oversight of development of the anti-HIV screen. Verbatim transcripts of the major three meetings of that committee during 1987-1990 were similarly recorded (*35-37*); names of the committee members are available in the transcripts. This committee met concurrently with the Harrap committee at its 1989 meeting.

For the anticancer screen application, there were two especially troublesome problems encountered with the tetrazolium assays that eventually prompted the development of a third alternative microculture assay method. For either MTT or XTT, tetrazolium reduction was dependent on the cellular generation of NADH and NADPH. This raised concern about the influence of glucose concentration on the formation of the colored tetrazolium formazan, which was measured colorimetrically as an estimate of cellular growth or viability. Studies with MTT indicated that a progressive reduction in MTT specific activity (MTT formazan formed/pg cell protein), which was observed during the course of a typical 7-d assay, was paralleled by a progressively decreasing glucose concentration (*19*). For XTT, there was a further problem resulting from the additional requirement of an electron transfer reagent, phenazine methylsulfate (PMS), to promote adequate cellular reduction of the tetrazolium. With XTT/PMS, variations in pH of the standard growth medium (RPMI-1640), typically caused by temporary removal of culture plates from the relatively high 5% CO_2 incubator environment, resulted in occasional formation of a crystalline material causing erratic optical density measurements. Crystal formation occurred in the pH range of 7.0-9.0 and could be attributed to reaction of PMS with glutathione (*19*).

In an attempt to eliminate the pH instability problem, a new culture medium was developed (*20*). The medium had a stable physiological pH of 7.4 at normal atmospheric levels of CO_2 and derived its buffering capacity primarily from β-glycerophosphate. The new medium was optimized to facilitate growth in atmospheric CO_2 by inclusion of biotin, L-asparagine, pyruvate, and oxaloacetate for metabolic stimulation of intracellular CO_2 production. With either the MTT assay or a nontetrazolium assay (described below), similar dose-response curves were

obtained for various standard anticancer drugs against cell cultures maintained either in the new medium (PDRG basal growth medium) under ambient CO_2 or in RPMI-1640 under a 5% CO_2 environment. However, a decision was subsequently made, consistent with a specific recommendation of the Harrap committee, not to incorporate the PDRG medium into the new screen, but rather to consider an alternative end point assay that was not as dependent on the particular CO_2 environment.

In an attempt to identify a suitable, nontetrazolium assay for use in the in vitro primary drug screen, a series of protein and biomass stains were investigated (*21*). These included anionic dyes that bound to the basic amino acid residues of proteins, as well as cationic dyes that bound to the negative, fixed charges of biological macromolecules. Of all the reagents tested, sulforhodamine B (SRB) gave the best combination of stain intensity, signal-to-noise ratio, and linearity with cell number. SRB is a bright pink anionic dye that, in dilute acetic acid, binds electrostatically to the basic amino acids of TCA-fixed cells.

2.3. Selection of Assay Parameters and Methodology

Under in vitro assay conditions, exposure to an antitumor agent may decrease the number of viable tumor cells by direct cell killing or by simply decreasing the rate of cellular proliferation. Many in vitro assays of drug sensitivity typically employ relatively low initial cell inoculation densities (e.g., a few hundred cells/well) followed by relatively long continuous drug exposure times (e.g.,6-7 d or considerably longer than the doubling times of many tumor lines). Such a selection of assay parameters, although favoring the detection of antiproliferative effects (i.e., growth inhibition), might, however, obscure otherwise potentially interesting patterns of differential cytotoxicity (e.g., net cell killing). Moreover, with an antiproliferative or growth inhibition end point, cell lines with very short doubling times (e.g., leukemias) might appear hypersensitive in comparison to more slowly growing tumor lines (e.g., from solid tumors). Additionally, potential problems of nutrient deprivation, as well as practical limitations on the use of pulse drug exposures, might, in the course of an assay, necessitate removal and replacement of medium. On the other hand, a longer assay duration might facilitate the detection of activity of relatively insoluble compounds or active trace constituents in mixtures or extracts. Furthermore, the longer assay format might be essential for detection of agents that required several cell cycles for expression of lethal drug effects.

An alternative selection of assay parameters was considered in order to enhance the screen's ability to discern interesting differences in net cell killing (i.e., actual reduction of biomass) among the sensitive panel lines. This required the use of a relatively large initial cell inoculum (e.g., 20,000 cells/well), and a relatively short drug exposure/incubation time (e.g., 1-2 d). Optimal exploitation of this format required a high level of sensitivity and reproducibility of the assay methodology, and the capability to measure reliably the initial viable cell densities ("t_0" values) just prior to drug introduction.

There were reasonable arguments for and against selection of either of these two alternative sets of assay parameters, or some compromise in between. Indeed, we extensively investigated the impact of these parameters on the screen's performance and, not surprisingly, found that certain kinds of compounds yielded results that contrasted greatly, depending on the particular choice of assay parameters. However, for purposes of further studies with the pilot-scale screen, as well as for initiation of the full-scale screen, the high-cell-inoculum/short-assay

protocol was selected for routine use. This selection was based principally on the desire to minimize the effects of variable doubling times of the diverse cell lines in the panel, to optimize the chances of detection of cell-line-specific or subpanel-specific cytotoxins, and to minimize the chances of obscuring such activities by nonspecific antiproliferative effects. This choice of assay parameters was also emphatically endorsed by the Harrap committee, after much discussion, debate, and extensive review of the relevant available experimental data.

Given the above decisions concerning assay parameters, the optimal choice of a tetrazolium assay (e.g., MTT or XTT) vs the SRB assay had to be determined for the desired application to a large-scale screening operation employing simultaneously many diverse tumor lines. Pilot-screening studies (*22,23*) were performed on a common set of compounds using both MTT and SRB, along with the selected assay parameters. Under the experimental conditions employed and within the limits of the data analyses applied, the assays gave quite comparable results. However, the SRB assay had important practical advantages for large-scale screening. Although the SRB procedure was more labor-intensive (e.g., required multiple washing steps), it had the distinct advantage of a stable end point (i.e., not time critical, in contrast to either of the tetrazolium assays). Screening capacity, reproducibility, and quality control all appeared to be markedly enhanced by adoption of the SRB for the primary screen (*23*). Therefore, the SRB assay was used subsequently for all routine screening operations.

2.4. Cell Line Panel

The initial panel incorporated a total of 60 different human tumor cell lines derived from seven cancer types, including lung, colon, melanoma, renal, ovarian, brain, and leukemia. Selection of lines for inclusion in the panel required that they adequately met minimal quality-assurance criteria (testing for mycoplasma, MAP, human isoenzyme, karyology, in vivo tumorigenicity), that they were adaptable to a single growth medium, and that they showed reproducible profiles for growth and drug sensitivity. Mass stocks of each of the lines were prepared and cryopreserved; these stocks provided the reservoir for replacement of the corresponding lines used for drug screening after no more than 20 passages in the screening laboratory (*6,23,24*).

Although many of the lines were well known and had been widely used in research, the clinical histories and/or original tumor pathologies of many of the lines were incomplete or unavailable. All cell lines in the interim panel were nevertheless subjected to detailed, specialized characterizations (e.g., histopathology, ultrastructure, immunocytochemistry) to verify or determine tissue and tumor type (*25*). Moreover, parallel projects were launched for the acquisition of better and more diverse candidate cell lines, and for the development of new lines directly from surgical specimens or from nude mouse xenografts for which the corresponding clinical backgrounds were more complete. Special focus was placed on major cancer types (e.g., breast and prostate) that were not represented in the initial panel owing to unavailability of suitable lines.

2.5. Pilot Screening Operations: Standardization and Reproducibility

A pilot-screening operation (*6,23*) was initiated in which the panel lines were inoculated onto a series of standard 96-well microtiter plates on day 0, in the majority of cases at 20,000 cells/well, and then preincubated in absence of drug for 24 h. Test agents were then added in five, 10-fold dilutions starting from the highest soluble concentration, and incubated for a further 48 h. Following this, the cells were fixed in situ, washed, and dried. SRB was added, followed by further washing and drying of the stained, adherent cell mass. The bound stain was solubilized and measured spectrophotometrically on automated plate readers interfaced with personal computers, which, in turn, were interfaced to a central computer.

A series of approx 170 known compounds, comprising commercially marketed (NDA-approved) anticancer agents, investigational (investigational new drug application [INDA]-approved) anticancer agents, and other candidate antitumor agents (compounds previously approved by the NCI Decision Network Committee for preclinical development based on activities in prior screens) were selected for pilot screening studies (*6*). The repetitive screening of these prototype "standard agents" was aimed at providing a suitable data base from which a variety of novel approaches to data display and analysis could be explored. The "standard agent data base" was also the basis for calibration and standardization of the screen, for the assessment of reproducibility of the screening data, and for the development of procedures for quality-control monitoring (*6,27*).

2.6. Information Technology

Facilitating the above analyses were the development of the COMPARE pattern recognition methodology and the mean-graph display, which supported both visual and automated analyses of the differential activity profiles of agents tested against the 60-cell panel (*6,12,13,26,27*). The mean-graph profiles of standard agents were highly reproducible over time; for example, the characteristic mean-graph profile of a given standard agent could be shown by COMPARE to be highly correlated among separate screening runs of the same compound over many months (*6,27*).

2. 7. Review and Recommendation to Operational Status

In November of 1989, a pivotal review meeting (*5,32*) was held at the NCI-Frederick Cancer Research and Development Center in Frederick, MD, the site of the newly constructed screening facilities. The full current memberships of the Harrap committee and an additional ad hoc advisory subcommittee for the natural products program, the BSC, the NCAB, and the President's Cancer Panel were invited to review jointly in detail the progress of implementation of the in vitro screen, and to provide recommendations for further directions. The resulting consensus recommendation was that the feasibility, reproducibility, and calibration of the screen were sufficiently established that full-scale operation should be formally initiated as soon as possible (*5*). DTP staff responded accordingly, and full operational status of the screen was established shortly thereafter. Table 1 summarizes the assay protocol and parameters for the operational screen as launched in 1990. Table 2 summarizes some of the pertinent screening laboratory operations and logistics. The annual operational costs of the new in vitro primary screen were budgeted at approx $3 - million/yr, or approximately one-third the operational costs of the P388 in vivo primary screen that it replaced.

Table 1
Assay Protocol and Parameters for the NCI In Vitro Antitumor Screen as Initiated in 1990[a]
[a]Further details are available in refs. (*6,21,23,25*).

Cell line panel
60 Lines total
7 Subpanels initially (lung, colon, renal, ovary, melanoma, brain, leukemia)
Lines used at • 20 passages from master stock
Culture medium
RPMI 1640
5% Serum
Cell inoculation densities: 5000-40,000 cells/well (96-well microtiter plate)
Preincubation: 24 h (no drug)
Sample dilutions Routinely 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}M or as specified
Duplicates performed at all concentrations
T_0 and "no-drug" controls included
Drug incubation: 48 h
End point assay: Sulforhodamine B protein stain

3. OPERATION 1990-1995

3.1. Routine Screening Operations

For routine screening as initiated in 1990, each sample was tested in the 2-d, continuous-drug-exposure protocol using five, $\log_{10}$-spaced concentrations starting at an upper limit of 10^{-4}M (or 100 μg/mL for natural product extracts or fractions thereof) against all of the current 60 cell lines comprising the panel. Most crude extracts were initially "prescreened" using only a single concentration (100 μg/mL) against the entire 60-cell-line panel; extracts that produced ~ 50% net cell killing of • 3 of the panel lines were routinely selected for testing in the full screen.

Details of the particular cell lines comprising the original panel, and the individual inoculation densities and other assay features used initially in routine screening operations were as published (*23,25*). From December 1, 1992, a modified panel, in which 10 of the original cell lines were replaced by a selection of breast and prostate cancer lines, was employed. Details of the replacement cell lines, inoculation densities, and other aspects of routine screening operations as of end of 1995 were as published (*13*).

The testing of a sample in the full 60-cell-line screen yields a corresponding set of 60 dose-response curves. Figure 1A-D illustrates four contrasting sets of composite dose-response curves. In Fig. 1A, the particular test compound had essentially no effect on the growth or

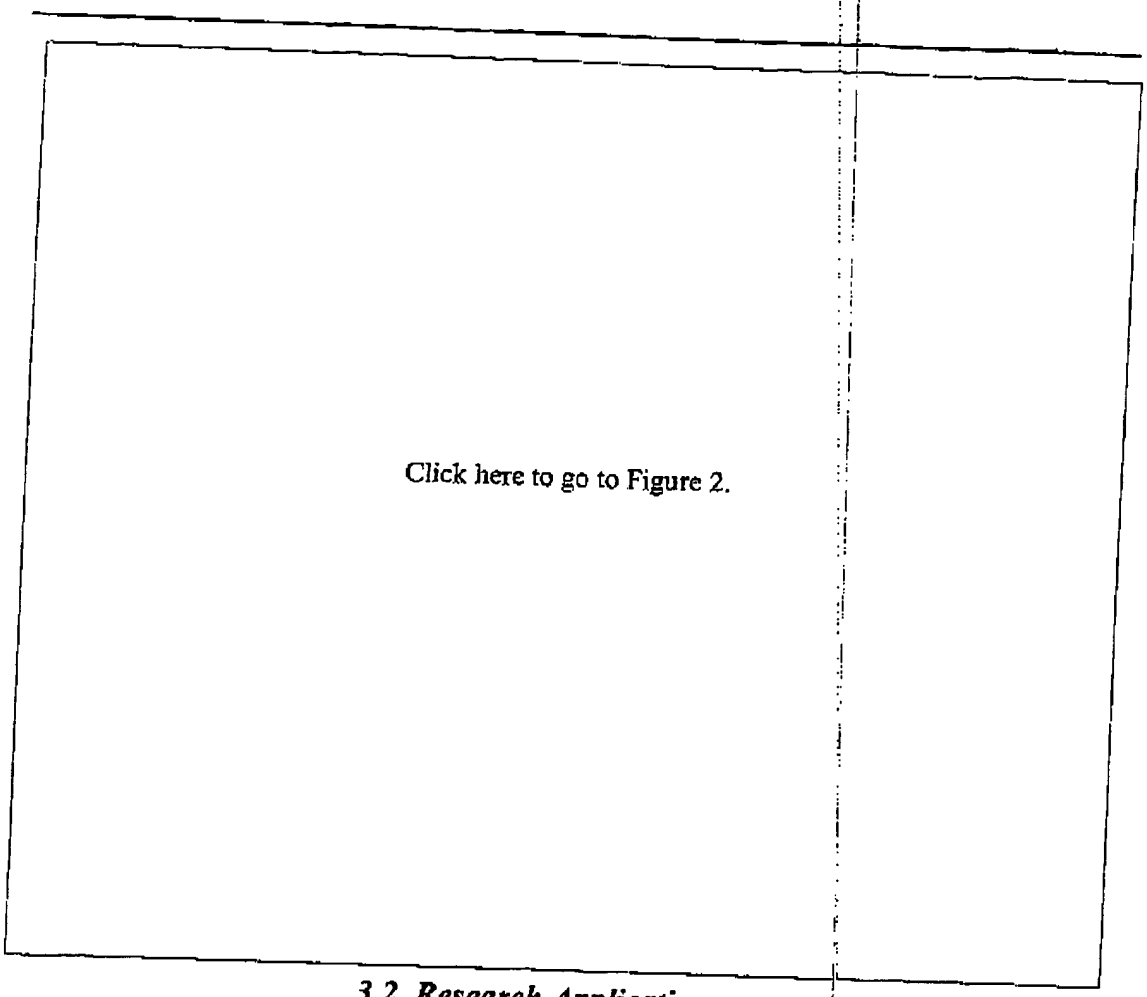
Table 2
Primary Screening Laboratory Operations and Logistics as Initiated in 1990[a]

Laboratory
8051 ft² total space
2 Floors
4 General support modules
20 Screening modules
50 Laminar-flow hoods
Staffing
44 Technicians
2 Senior Supervisors (Ph.D.)
Cell inoculations/drug additions
6 Lines assigned per technician
3 Lines/2 compounds/96-well plate
Colorimetric end-point determinations
10 Plates/compound
4000 Plates/wk
12 Automated plate readers
Quality control
Manual
Automated
Computer support: 20 in-lab PCs networked to central computer
Calibration/standardization of screen
Daily standards
Monthly standards
Standard agent database

[a]Further details are available in refs. (*6,23,27*).

viability of any of the 60 cell lines. Figure 1B shows the effect of a test compound that was cytotoxic, although with essentially equivalent potency to all of the panel lines. Neither of these screening results is particularly useful. In contrast, Fig. 1C illustrates results from a compound showing pronounced cytotoxicity, although with considerably divergent potencies against the individual cell lines. Screening profiles, as exemplified by Fig. 1C, which manifest "differential" growth inhibition and/or cytotoxicity have been of particular interest as the basis for research applications of the screen, as well as for the selection and prioritization of compounds for in vivo evaluation. Figure 1D shows the characteristic dose-response composite profile from a highly potent natural product, dolastatin 10 (Fig. 2; see also refs. *38,39*); there appears to be a marked degree of differential growth inhibition and/or cytotoxicity among the various panel lines. However, for any given line, the inhibitory effect of the compound, within the tested concentration range, is not concentration-dependent. A more detailed analysis and explanation of the basis for this type of profile is provided elsewhere (*13*). Critical to any research application of the screen is the remarkably high degree of reproducibility of the screening profile of a given compound tested repetitively over time (*6,13,27*).

Click here to go to Figure 1.



3.2. Research Applications

NCI staff, collaborators, and others have been exploring diverse data analysis strategies and methods with data generated by the in vitro screen. Reviews and other publications describing such studies are available (*6,12,13,40-44,48*). As discussed recently (*13*), the appealingly simple mean-graph and COMPARE analysis methodologies provide useful support for a number of research applications. Such applications encompass the discovery of new members of known mechanistic classes. For example, the mean-graph screening profiles (not shown) of halichondrin B (*45,46*) and spongistatin 1 (*47*) (Fig. 2) were revealed by COMPARE to resemble closely the mean-graph profile (not shown) of dolastatin 10 (*38,39*) (Fig. 2) and other known members (e.g., vinca alkaloids, taxol, rhizoxin, maytansine) of the general class comprising tubulin-interactive antimitotics (*48*). Follow up biochemical studies (*49,50*) confirmed the general antimitotic mechanism anticipated from the initial evaluation of halichondrin B and spongistatin 1 in the 60-cell screen.

The counterpoint to discovery of new members of known mechanistic classes is the discovery of new antitumor mechanistic classes and new members therein. Such application of the 60 cell screen is exemplified by studies of 9-methoxy-N^2-methylellipticinium acetate (MMEA) and related ellipticinium derivatives. MMEA produced an unprecedented screening profile, in which the brain tumor cell line subpanel showed consistently higher sensitivity to MMEA cytotoxicity than did other lines comprising the panel (*6,51*). Subsequent studies revealed a high correlation between uptake and accumulation of MMEA, and/or metabolite(s) thereof, and MMEA cytotoxicity in the sensitive brain tumor cell lines (*52*). Both uptake and cytotoxicity of MMEA were blocked by reserpine. Other experiments further suggested a resemblance of the MMEA transporter in the brain tumor lines to a constitutive biogenic amine transport process characteristic of certain glial elements of normal brain (*52*). Recent in vivo studies of the 9-chloro analog of MMEA have shown evidence of in vivo antitumor activity against an intracranially implanted brain tumor cell line (53).

Another novel lead that shows an unprecedented profile in the 60 cell-line screen is cephalostatin 1 (*54,55*) (Fig. 2). Figure 1C is from the screening of this compound. Figure 3 shows the GI_{50}, TGI, and LC_{50} mean graphs, constructed from the data of Fig. 1C, and defined in detail elsewhere (*13*). COMPARE analyses were performed, using described procedures (*13*), with the mean-graph profiles of cephalostatin 1 as the "seed" against a screening data base from approx 40,000 structurally diverse compounds. When the profiles were ranked in order of degree of similarity to the seed, the top-ranking 13 profiles were all found to be derived from prior tests of cephalostatin 1 or other closely related members of the cephalostatin series (*55*). On the other hand, the characteristic screening profile of the cephalostatins did not show comparable correlations to any member(s) of the standard agent data base, suggesting that the differential cytotoxicity of this lead derives from an unprecedented, but as yet undefined, mechanism of action.

Another important research application has become apparent from studies of structure-activity relationships (SAR) and chemical analog synthesis. In these applications, the NCI in vitro screen provides an opportunity for lead optimization based on the feedback of both quantitative and qualitative biologic data. For example, members of a chemically related series can be compared not only with respect to relative potencies, but also with respect to the degree to which they do or do not retain the desired cell line specificity or subpanel activity of the lead compound. Research applications of this nature are illustrated by recent studies (*51*) with the aforementioned ellipticinium series and also by SAR investigations related to the novel antitumor lead, halomon (*56,57*).

An exploratory research application of the screen has been pursued intramurally at NCI for the selection and bioassay-guided fractionation of natural product extracts from the NCI repository. The screen has been used either to select or, alternatively, to eliminate from further consideration extracts having screening profiles either similar to or distinctly different from any known standard agent or mechanistic class. Bioassay support for fractionation has employed either the full 60 cell screen or one or a few individual cell lines selected on the basis of the 60 cell screening profile. As of end of 1995, approx 80,000 extracts had been screened against the 60 cell panel, however, the majority of these were only in the single-concentration "prescreen" protocol. Extracts failing the initial prescreen-select criterion were not subjected to further

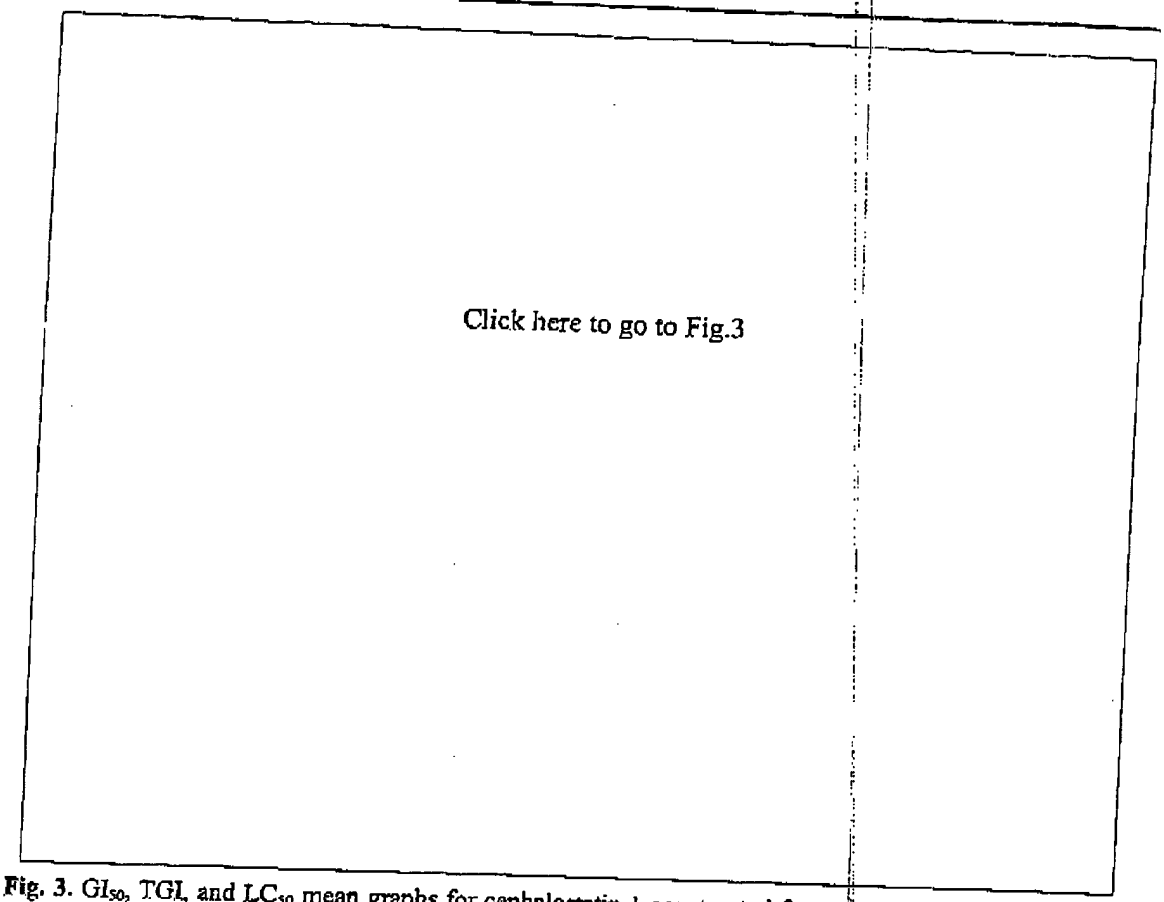


Fig. 3. GI_{50}, TGI, and LC_{50} mean graphs for cephalostatin 1 constructed from the data illustrated in Fig. 1C. Response parameter definitions, methods of constructions and interpretation of mean graphs have been reviewed recently in detail elsewhere (see ref. *13*). The tumor cell line subpanels are identified as follows: I (leukemia); II (lung, non-small-cell); III (lung, small-cell); IV (colon); V (brain); VI (melanoma); VII (ovary); VIII (kidney). These mean-graph "fingerprints" can be shown by pattern-recognition analyses (e.g., see methods of COMPARE analyses also reviewed recently in ref. *13,44*) to be highly correlated with those of other cephalostatins; in contrast, they were not similarly correlated with any of the "standard agents" (*6,13*).

testing; as a result, the data base of natural products screening using the full, five-concentration assay against the 60 cell panel is not nearly as extensive as the data base derived from the full screen evaluation of pure compounds. Nevertheless, several hundred extracts having novel screening profiles were identified, and studies have been initiated to isolate, identify, and characterize further the individual active constituents.

There are other emerging research applications of the NCI in vitro screen aimed at exploiting advances in knowledge of tumor biology and the molecular genetics of cancer. For example, experimental measurements of the differential expression in the panel cell lines of potential cell growth regulatory and/or drug sensitivity or resistance determinants are being

used to construct hypothetical mean-graph profiles, which are then used to search the available data bases for compounds that produce actual screening profiles similar to the desired hypothetical one(s). For instance, a hypothetical mean-graph fingerprint constructed from quantitative expression values for the mdr-1/ P-glycoprotein in each of the panel cell lines was used as the seed for COMPARE analyses (*58*). A series of compounds was thereby identified having screening profiles highly correlated with the constructed probe. Subsequent biochemical analyses confirmed that the selected compounds were indeed substrates for the P-glycoprotein. In a related study (*59*), comparably high correlations were found for the same compounds with respect to a probe constructed of rhodamine efflux values, which are functional assay counterparts of mdr-1 expression. NCI staff and collaborators are exploring similar research strategies with numerous other potential sensitivity or resistance determinants, such as oncogene or tumor suppressor gene products, growth factor receptors, transporters, and the like.

3.3. Lead Discovery and Development

Research applications notwithstanding, the ultimate value of the NCI in vitro screen will and should be judged by the extent to which it uniquely contributes to the discovery and development of new clinically useful anticancer drugs. As summarized elsewhere by Sausville (Chapter 11, this volume), from the tens of thousands of pure compounds tested initially in the in vitro primary screen, a subset was selected for in vivo preclinical follow up. By the end of 1995, several of these compounds, which arguably were selected for NCI development based principally on their novel bioactivity profiles in the in vitro screen, were expected to achieve INDA approval by the FDA for phase I clinical trials. As also described separately by Plowman et al. (Chapter 6, this volume), continuing innovations and refinements of the in vivo preclinical followup evaluation of leads selected by the in vitro screen may both improve and accelerate discovery of the most novel and promising new leads for drug development.

4. CONCLUSION

It has been more than a decade since the concept for the NCI in vitro primary screen was first proposed. The initial 5 yr, 1985-1990, were consumed with developing key elements of the screening model, designing and constructing the physical facilities to accommodate the screening operations, recruiting and training staff, implementing and evaluating a pilot-scale screen and data management operations, and calibration and standardization of the screen. During the subsequent 5 yr of operation of the screen, 1990-1995, more than 100,000 materials, including pure compounds as well as natural product extracts, were tested either in the prescreen and/or the full screen assay against the 60-cell panel. The accrued data bases have provided a rich source of information that has proven to have considerable utility in certain research applications. Ongoing and future studies will likely reveal additional important research applications of the data accumulated to date. However, the prudence of continuing the 60-cell panel screening operations indefinitely into the future can appropriately be questioned. The decade of 1985-1995 was also one of exciting, if not explosive, progress in the understanding of the molecular genetics of cancer. This realization has been a stimulus to pursue the "molecular characterization" (see Sausville, Chapter11, this volume) of the cell lines of the NCI screen with respect to selected genes, gene products, and other possible "molecular targets" contributing to

maintenance or reversion of the malignant phenotype. It is hoped that such information will facilitate use of the NCI 60 cell panel in vitro screen, and/or the accrued screening data bases therefrom, to discover novel molecular target-directed leads. Whether these and other potential research applications of the screen are sufficient to justify continuation is an appropriate matter for informed debate and consensus. Validation of any past, present, or future application of the NCI 60 cell screen as an effective tool for discovery of clinically useful new antitumor drugs, must await definitive clinical evaluation of new investigational agents whose discovery was uniquely dependent on the screen. Realistically that may require another decade into the future.

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APPENDICES

Appendix A. Participants (Non-NCI) in NCI Workshop on "Disease-Oriented Antitumor Drug Discovery and Development," January 9-10, 1985, Bethesda, MD

Michael Alley[a]
Ghanem Atassi
Bruce Baguley
Laurence Baker
Ralph Bernacki
Bijoy Bhuyan
Arthur Bodgen
Jerry Boyd
William Brodner
Martin Brown
James Catino
John Johnston
Michael Johnston
John Kovach
Victor Levin
Daniel Martin
Patrick McGovern
Christopher Mirabelli
John Montgomery
Franco Muggia
Ronald Natale
Robert Newman

Thomas Corbett
Daniel Dexter
Benjamin Drewinko
Gertrude Elion
Mortimer Elkind
Edward Elslager
David Ettinger
Oystein Fodstad
Martin Forbes
Henry Friedman
David Gillespie
David Goldman
Robert Goodman
Michael Grever[b]
Daniel Griswold
Ladislav Hanka
Kenneth Harrap
David Hesson
Susan Horwitz
David Houchens
Peter Houghton
Robert Jackson
Randall Johnson
Kent Osborne
George Pettit
Theodore Phillips
Alexander Pihl
Mark Rosenblum
Alan Rosenthal
Marcel Rozencweig
Youcef Rustum
Clifford Schold
John Schurig
Alan Shefner
Robert Sutherland
Raymond Taetle
Ken Tew
Richard Tuttle
David Van Echo
Daniel Von Hoff
George Weber
Larry Weisenthal
Thomas WiliaTns
Benjamin Winogard
Charles Young

[a]Michael Alley subsequently joined the staff of DTP-NCI in 1987.
[b]Michael Grever subsequently joined the staff of DTP-NCI in 1990.

Appendix B
Members of Ad Hoc Review Committee for NCI In Vitro/In Vivo Disease-Oriented Screening Project

Bruce Baguley
Ralph Bernacki
Arthur Bogden
Michael Buas
Thomas Carey
Thomas Connors
Thomas Corbett
Mortimer Elkind
John Faulkner[b]
Isaiah Fidler
Heinz-Herbert Fiebig[c]
Oystein Fodstad
James Goldie
David Goldman
Daniel Griswold
Kenneth Harrap[d]
Sidney Hecht[b]
Susan Horwitz
Peter Houghton George Pettit[b]
Alexander Pihl
Alan Rosenthal
Sydney Salmon
Phillip Skehan[a]
Raymond Taetle
Peter Twentyman
Larry Weisenthal
Robert Whitehead

[a]Phillip Skehan served on the Committee prior to his joining the staff of DTP-NCI in 1987. [b]John Faulkner, George Pettit and Sydney Hecht comprised an ad hoc advisory subcommittee in 1989 for the natural products program. [c]Heinz-Herbert Flebig served as a visiting scientist with DTP-NCI in 1988-89. [d]Kenneth Harrap served as Chair of the Committee from its inception in 1985 through its last meeting in 1989.

Appendix C
Participants (Non-NCI) in NCI Workshop on "Selection, Characterization and Quality Control of Human Tumor Cell-Lines for the NCI's New Drug Screening Program," May 27-28, 1987, Bethesda, MD

Bruce Baguley	Steven Jacobs
Ralph Bernacki	Edward Keenan
June Biedler	James Kozlowski
Arthur Bogden	Chung Lee
Michael Brattain	Al Liebovitz
Thomas Carey	John Masters
Desmond Carney	Stanley Mikulski
Thomas Corbett	Mary Pat Moyer
Joseph Eggleston	Charles Plopper
Janine Einspahr	Mark Rosenblum
Howard Fingert	Phillip Skehan[a]
Oystein Fodstad	Robert Sutherland
Ian Freshney	Raymond Taetle
Eileen Friedman	Peter Twentyman
Susan Friedman	John Wallen
David Goldman	Michael Wiemann
Daniel Griswold	Larry Weisenthal
Kenneth Harrap	James Willson
Robert Hay	Bernd Winterhalter

[a]Phillip Skehan subsequently joined the staff of DTP-NCI (see footnote a, Appendix B).